Bridging the Gap between Muscle and Bone: Examining Tendon Identity and Development.

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INTRODUCTION

- Tendons are fibrous connective tissues that connect skeletal muscle to bone[1].
- Tendons function to transmit force from muscle to bone to facilitate locomotion[1].

Fig 1. Patellar Tendon Structure

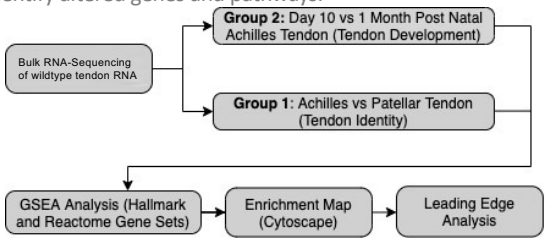
- The tendon extracellular matrix is composed of type I collagen as well as other minor collagens and proteoglycans[2].
- There are over 4000 tendons in the human body[1]; however, the molecular pathways that define different tendons is largely unknown.
- Despite their important role in motor coordination and posture, the molecular pathways involved in tendon developments remain to be fully elucidated.

OBJECTIVES

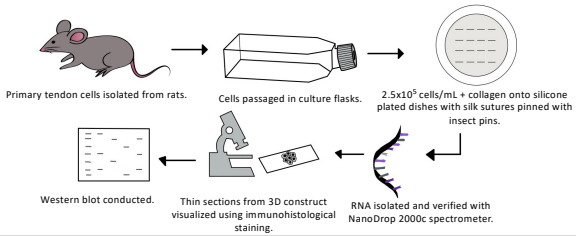
1. Analyze RNA-seq data to identify pathways involved in tendon development and identity
2. Validate findings using tissue-engineered tendon bundles.

METHODS

Part 1: Using RNA-seq data previously generated in the lab to identify altered genes and pathways.



Part 2: 3D Tendon Construct Protocol.



RESULTS

Gene sets involved in cellular metabolism, cell signaling, and inflammation are increased in Achilles tendons at 1-month

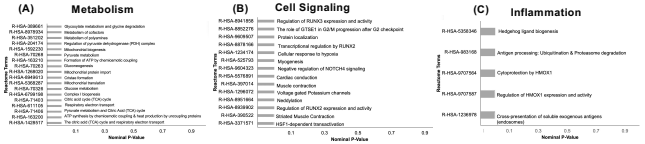


Fig 2. GSEA using the reactome dataset revealed that (A) Metabolism, (B) Cell Signaling, and (C) Inflammation terms were enriched in 1-month Achilles tendons compared to 1-month patellar tendons. Data are plotted according to their adjusted nominal p-value, with all having a $p < 0.0025$. n = 3 mice for Achilles tendon, 2 mice for patellar tendon.

Pathways related to muscle contraction, skeletal cell differentiation, and conductance are increased in 1-month Achilles tendons

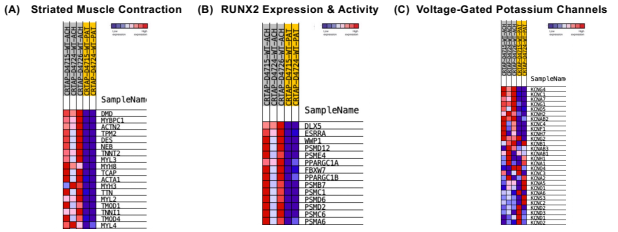


Fig 3. Heat maps generated following GSEA analysis. Specific Reactome gene sets examined include (A) Striated Muscle Contraction, (B) RUNX2 Expression and Activity and (C) Voltage-gated potassium channels.

Metabolic, purinergic, and inflammatory pathways are increased in 1-month Achilles tendons compared to post-natal day 10 (P10)

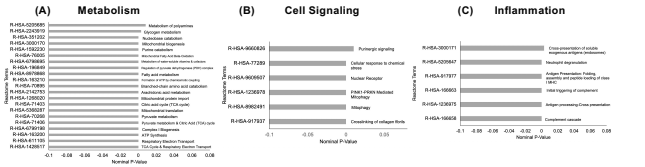


Fig 4. GSEA using the reactome dataset revealed that (A) Metabolism, (B) Cell Signaling, and (C) Inflammation terms were enriched in 1-month Achilles tendons compared to post-natal day 10 (P10). Data are plotted according to their adjusted nominal p-value, with all having a $p < 0.015$. n = 3 mice for each group.

Genes of interest include collagen fibers, and P2X receptors.

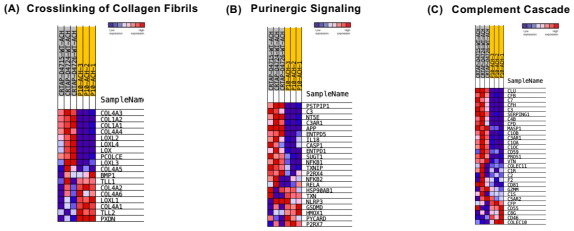


Fig 5. Heat maps generated following GSEA analysis. Specific Reactome gene sets examined include (A) Crosslinking of Collagen Fibrils, (B) Purinergic Signaling and (C) Complement Cascade.

DISCUSSION

Achilles vs. Patellar Tendon Findings

- Given that skeletal muscle is striated muscle and forms the basis of the Achilles tendon and patellar tendon links two bones, it is understandable that the Achilles tendon has greater expression of genes involved in striated muscle contraction[1].
- There is a greater expression of RUNX2 and DLX5 in rats exposed to greater mechanical stretch, which may indicate that there is Achilles tendon experiences more mechanical stretch than in the Patellar tendon[5].
- Diseases involving mutations of potassium channels are often associated with reflex conditions, which may be related to alterations in the golgi tendon organ.

1 Month vs Day 10 Findings

- Cross-linking of collagen fibrils increases with development[6]. One study proposes that fully cross-linked mature fibrils would appear beyond 19 days of tendon development[6].
- The complement cascade is involved in many stages wound healing, and tend to be major cellular players in tendon, ligament, muscle and bone healing[7]. Given that normal healing takes 12 weeks, it is to be expected that these markers are increased in the later days of development[7].
- The release of ATP and binding to the P2X receptors is often associated with an injury response[8]. Like the complement cascade, a greater expression 1 month after birth may be indicative of modifications to the tenocytes and ECM.

NEXT STEPS

1. Continue with 3D Tendon Construct experimental protocol.
2. Analyze findings of 3D tendon culture and cross listing them with the findings of the bulk RNA-seq analysis.

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